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THREE FACTORS AFFECTING LARVAL CHOICE OF FOOD PLANT[1]

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PRIOR TO THE INITIATION of comprehensive tests of the attraction by larvae of *Pieris rapae* of various food plants, a number of preliminary trials were made to test the effect of external factors which might influence the correctness of these tests. Of the factors which were thought most important, the following were selected for trial:

(1) Influence of direction of air movement ("wind").
(2) Influence of age (size) of larvae.
(3) Influence of food previously eaten by larvae, or strain of larvae.

EFFECT OF WIND MOVEMENT

An experiment devised to test this factor was set up as follows: A nursery flat about 24 inches square was filled with vermiculite. Pots of plants, each about the same size, were placed in the vermiculite spaced about as shown in Figure 1. The table under the flat was marked A, B, C and D. The direction of the wind movement was at side B. In the tests, larvae were placed in the central area, shown by a small circle and allowed to go to the plants, or to leave the flat.

Ten different larvae were used for each experiment, and these were used a total of ten times each. Thus, each experiment involved 100 trials.

The wind direction relative to the position of the plants was changed by turning the flat by 90° after each 100 trials, as shown by the illustration (Fig. 1). The first position tested was with mustard toward the dirction from which the breeze came (side B). The results of the preferential selection by the larvae are shown on Table 1.

Kale was selected by 19% of the larvae, mustards by 56%, radish

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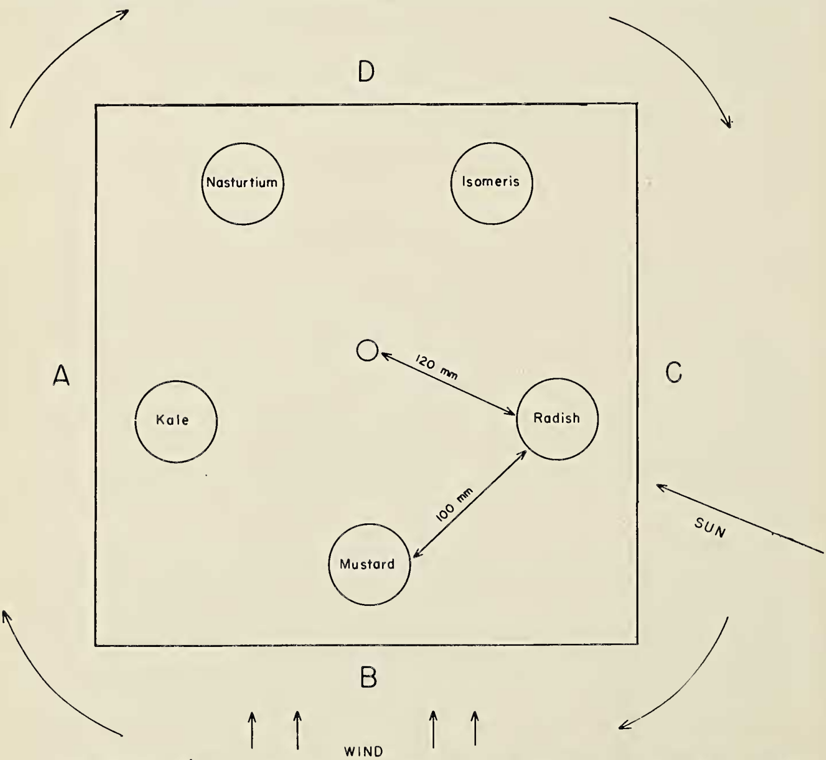
TABLE 1. The effect of wind movement on the choice of food plants by larvae of *Pieris rapae*

	Kale	Mustard	Radish	Nasturtium	Isomeris	None	Total no. of trials
	A 19	B 56	C 18	D 3	D 1	3	100
90°	D 20	A 20	B 52	C 4	C 2	2	100
90°	C 43	D 25	A 26	B 2	B 0	4	100
90°	B 53	C 15	D 13	A 8	A 5	6	100

Ten 14.1 mm to 15.7 mm kale-bred larvae of *Pieris rapae* were used per test with each test ten times at each position. The parents of the larva were collected at western Orange County in a cabbage field.

by 18%, nasturtium by 3% and Isomeris by 1%. Larvae which left the flat without selection amounted to the additional 3%.

With the flat turned 90° so that the radish was now in position B (in the direction of the wind), the selection results were very different. Kale remained relatively unchanged at 20%, mustard dropped from



1. The set-up for the selection experiments. The arrows indicate the direction of turning of the nursery "flat." The direction of wind and sun are indicated.

56% to 20%, radish rose from 18% to 52% at position B, while the remainder were unchanged.

With a further 90° turn in the direction of the arrows (Fig. 1), the radish plant was now opposite the direction of the wind, and nasurtium-Isomeris was toward the wind. Kale at position C to the right side now rose from 20% to 43%, mustard at position D (away from the wind) rose slightly and was now 25%, radish at the left side was now 26% and nasturtium-Isomeris (toward the wind) was still relatively unchanged at 2%.

The final 90° turn placed kale in the position toward the wind. Here, 53% of the larvae went to kale, 15% to mustard (right side), 13% to radish (opposite side), and 13% to nasturtium-Isomeris (left side).

CONCLUSIONS:

(1) Of the four sides of the flat which could face the direction of the wind, the plants on that side were favored in three of the four arrangements, namely, mustard, radish and kale. Only when the nasturtium-Isomeris combination was toward the wind was the frequency in position B not increased. In fact, at that time, it was *decreased.*

(2) These tests, of course, cannot indicate which of the three favored plants are relatively more favored since they each received about the same percentage of selection when toward the wind. However, when away from the wind, radish received fewei selections than either kale or mustard.

(3) The Isomeris-nasturtium combination received its highest selection when in position A (left side). In this position also, more larvae left the flat without a plant selection than in any other position.

(4) The higher selection of kale (43%) while at position C (right side), than radish (26%) while at position A (left side) seems to indicate a preference for kale rather than for radish.

(5) The lowest selection for the nasturtium-Isomeris combination was when it was in position B (toward the wind). This would seem to indicate that these plants (and Isomeris especially) repel rather than attract larvae of *Pieris rapae.*

(6) The data indicate that the direction of wind movement is of great importance in larval selection of food plant, since in all cases where the plant has a positive effect on selection, that position in the direction of the wind has by far the greatest selective influence. It is apparent that the reverse is also true, that if a plant is repellent, the effect is greatest when the wind comes from that direction.

[NOTE: The differences between the figures indicated are so great that the element of chance being involved is negligible. A chi-square test on the first trial gives a probability of less than one in a million that the differences are due to chance alone.]

EFFECT OF LARVAL SIZE ON PLANT SELECTION

Tests for the determination of influence of larval size on plant selection were made in the same manner as indicated previously, except that all tests were carried out in a room with no wind or breezes. Nevertheless, the flat was turned 90° three times for each test. This applies to all subsequent tests for the sake of safety. Two series of tests were made, using larvae of different origin.

The first series of tests (Table 2) was carried out with the use of kale-bred larvae. Larvae of different ages and sizes (size is roughly proportional to age) were separated and measured. Four larvae were isolated, these being the following sizes: : 8.4, 14.7, 19.0 and 21.0 mm in length. The tests were carried out by five trials of a given larva at each position with respect to the direction of the wind (Fig. 1), giving a total of 20 trials for each size larva.

TABLE 2. Choice of five kinds of plants by different sized larvae of *Pieris rapae* (Series 1)

Size of larvae (mm)	Kale	The number of times and percentage larva goes to Mustard	Radish	Nasturtium	Isomeris	None	Total Number of trials
21.00	16—80%	4—20%	0— 0	0—0	0—0	0—0	20
19.00	13—65%	3—15%	3—15%	1—5%	0—0	0—0	20
14.70	11—55%	4—20%	4—20%	1—5%	0—0	0—0	20
8.40	10—50%	5—25%	5—25%	0—0	0—0	0—0	20

One single kale-bred larva was used for each size; tested each larva five times at each position.

The 8.4 mm larva selected kale 50% of the time, radish 25% of the time and mustard 25% of the time.

The 14.7 mm larva selected kale 55%, mustard 20%, radish 20% and nasturtium 5% of the time.

The 19.0 mm larva selected kale 65%, mustard 15%, radish 15% and nasturtium 5% of the time.

The 21.0 mm larva selected kale 80% and mustard 20% of the time and no others.

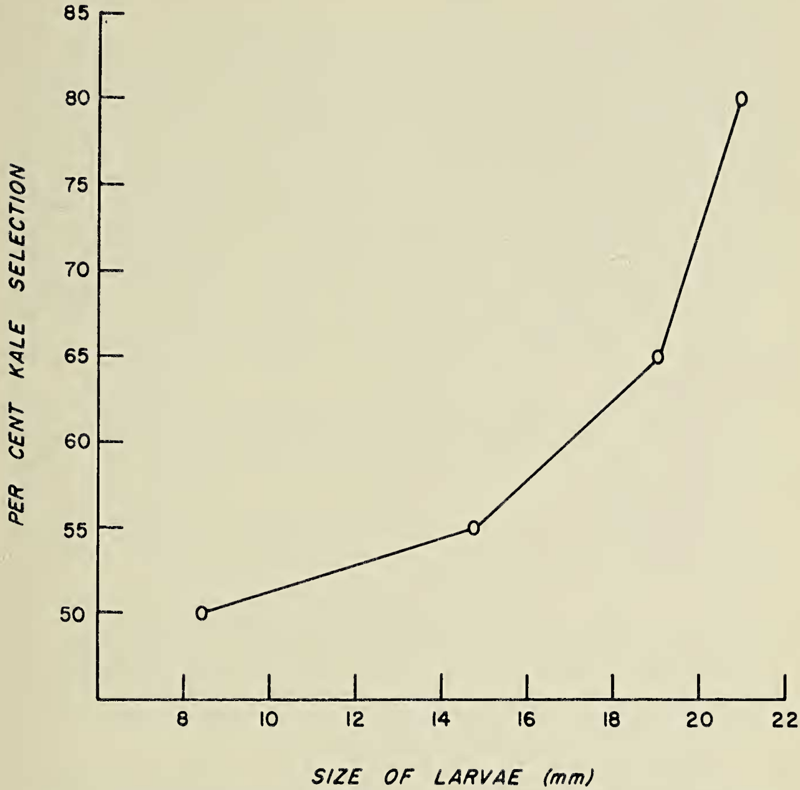
The probability, as caculated by chi-square, that the differences as indicated might be due to chance are less than 1/million, 1/100,000, 1/500, 1/500 respectively for each experiment.

CONCLUSIONS:

(1) The increase in selection of kale for each experiment is correlated with increase in size of the larvae (Fig. 2).

(2) Kale is the plant selected over all the others in this experiment. It should be noted that the larvae had been bred on kale prior to the experiment, and previous generations had been bred on cabbage.

(3) Not one larva went to Isomeris. This is interesting in view of the observation in the preceding experiments that Isomeris was



2. The relation between size (and age) of larvae and the percentage of the time kale was selected. The size of the larvae is directly correlated with an increase in selection of kale.

repellent to *Pieris rapae* larvae.

(4) The older or larger the larvae, the greater the selection of kale over all other plants (Fig. 2). The relationship is not a straight-line, but rather a geometric increase. This indicates that there is an increase in the critical powers of food perception in the older or larger larvae.

In the Series No. 2 tests (Table 3), larvae were used which had previously been grown on nasturtium. They were obtained from Ventura County on nasturtium and were continued in the laboratory on that plant.

Two larvae 22.4 mm in length, and four larvae 16.5 mm in length were used in this experiment. Otherwise, the trials were managed in the same way as in previous experiments with the exception that the total number of trials was 80 for large larvae and 160 for the

smaller larvae.

The selection by these larvae bred on nasturtium (Table 3) was radically different from the selection by larvae bred on kale (Table 2). The selections of kale by the small larvae bred on nasturtium were 15% as compared with 55% for a comparable size bred on kale. The selections of kale by the large larvae bred on nasturtium were 22% compared with 80% for a comparable size bred on kale. The greatest shift in food plant selections for the nasturtium-bred small larvae was 49% to mustard as compared with 25%, 9% to nasturtium as compared with 0%, and 16% with no selection as compared with 0%. For the large larvae, comparable data were 34% to mustard as compared with 20%, 33% to nasturtium as compared with 0%, and 9% with no selection as compared with 0%.

CONCLUSIONS:

(1) The shifts in food-plant-selection by the larvae grown on different plants are highly significant and indicate a direct relationship between the feeding habits of the larvae, and their selection when given a free choice.

(2) The direct relationship between the size (and age) of the larvae and their selection of the plant previously eaten is clearly indicated.

(3) A plant previously of nearly negligible selection may be the most highly selected if the larvae have been grown on that plant. For example, larvae selected nasturtium over all others (33%) after being bred on that plant (Table 3), whereas larvae bred previously on kale had almost no selectivity for that plant.

INFLUENCE OF PREVIOUS FOOD PLANTS OF LARVAE

Additional experiments to test the influence of previous exposure to larval food plants are reported on here.

The first comparative test involves two strains of *Pieris rapae,* one from a population growing in the wild on black mustard and the other growing on cabbage. These strains were kept in the laboratory for several generations; the mustard strain was bred for over six generations on mustard in the laboratory and the kale strain was bred on kale in the laboratory for over ten generations.

Larvae were selected for these experiments which had a size of 14 to 16 mm. Using twenty-five larvae of each strain, 600 tests of selection were made (Table 4): the selected plants were mustard, kale, nasturtium, Isomeris and Cleome.

The only significant differences detected in the tests between the two strains were the relative selections made of mustard and kale. Larvae from the mustard-bred strain selected mustard 61% of the time compared with the kale-bred strains of larvae of 24% on that plant. On the other hand, kale was selected by the mustard-bred larvae 20%

TABLE 3. Choice of five kinds of plants by different sized larvae of *Pieris rapae* (Series 2)

Size of larvae (mm)	The number of times and percentage larvae go to: Kale	Mustard	Radish	Nasturtium	Isomeris	None	Total number of trials	No. of larvae
22.4	18—22%	27—34%	1— 1%	26—33%	1—1%	7— 9%	80	2
16.5	24—15%	79—49%	16—10%	14— 9%	1—0.7%	26—16%	160	4

nasturtium-bred larvae were used in these tests; they were collected in Ventura County on nasturtium plants and fed in the laboratory on nasturtium. 25 different, 14 to 16 mm larvae were used for each test.

TABLE 4. Food plant choice by larvae of *Pieris rapae* previously fed on different food plants (Series I).

Type of larvae	The number of times and percentage larvae go to: Mustard	Kale	Nasturtium	Isomeris	Cleome	None	Total Trials
Mustard-bred	364—60.7%	119—19.8%	56—9.3%	18—3.0%	12—2.0%	31—5.2%	600
Kale-bred	144—24.0%	354—59.0%	50—8.3%	21—3.5%	15—2.5%	16—2.7%	600

Mustard-bred larvae were collected in the Arboretum and raised on black mustard in the laboratory for over six generations. Kale-bred larvae were collected in western Orange County on cabbage and raised on kale in the laboratory for over 10 generations.

TABLE 5. Food-plant choice by larvae of *Pieris rapae* previously fed on different food plants (Series II).

Type of larvae		The number of times and percentage larvae go to: Mustard	Kale	Nasturtium	Isomeris	Radish	None	Total number of trials
Nasturtium-bred	(2)	28—35.0%	10—12.5%	12—15.0%	1—1.3%	11—13.9%	18—22.3%	80
Kale-bred	(2)	18—22.5%	39—48.8%	3— 3.8%	0—0	18—22.5%	2— 2.5%	80

The nasturtium-bred larvae were from Ventura County, collected in a nasturtium garden. The parents of the kale-bred larvae were collected in western Orange County on cabbage; the larva are the first generation fed on kale.

of the time as compared with 59% by the kale-bred larvae. Testing these differences by chi-square using the four-fold table indicates a probability of less than 1/million that the differences could be due to chance alone. This may be calculated in another way, that is, by taking $364 + 354 = 718$ for one value and $119 + 144 = 263$ for the other. The expectation that these differences might be due to chance alone, that is, that the deviations $\frac{718 - 981}{2}$, and $\frac{971 - 263}{2}$ are not due to chance is well over one in a million.

CONCLUSIONS:

(1) The shift in selection of mustard or of kale according to whether or not the larvae were from a kale or mustard strain is indicated by the experiments. The data are highly significant at an incredibly high level.

(2) There is no significant change indicated in the selective level of the other plants involved as to whether or not the larvae were from the mustard or kale strain.

The second series of data on the problem of food plant selections involves nasturtium-bred larvae from a nasturtium strain and kale-bred larvae from a cabbage strain (Table 5). The experiments were carried out as before, two larvae of each strain were used, the same size for a total of forty trials each, of which ten were in each position as indicated in Figure 1.

The differences between the plant selections are again highly significant. The nasturtium-bred larvae as compared with the kale-bred larvae preferred mustard (35% as compared with 22.5%) and nasturtium (15% over 4%). The kale-bred larvae preferred kale (49% over 12.5%) and radish (22.5% over 14.0%). Neither selected Isomeris to any extent. A high proportion (22%) of the nasturtium-bred larvae rejected all plants.

CONCLUSIONS:

(1) As in previous experiments, there was a shift in selection by the larvae according to the previous larval food plants.

(2) This shift in selection was in the direction of the plant they had previously fed on. For example, nasturtium-bred larvae selected nasturtium better than did kale-bred larvae.

(3) Even though nasturtium-bred larvae selected nasturtium better than did kale-bred larvae, mustard was preferred over nasturtium and over kale.

(4) The greater selection of mustard over kale by nasturtium-bred larvae is significant not only in this experiment but also in a previously indicated test (Table 3). In both cases, kale is poorly preferred as compared with mustard when the larvae were nasturtium-bred.

(5) Radish is of lower selective influence when the larvae are

nasturtium-bred than when they are kale-bred. This was indicated also in previous tests (Tables 3 and 1).

(6) A significantly higher percentage of rejects (22%) are made by the nasturtium-bred larvae than by the kale-bred. This was also indicated in previous tests (Table 3 as compared with 1, 2 or 4).

DISCUSSION

All three factors, investigated as to their relevance in influencing larval choice of food, have been shown to be important in that regard. These factors are:

(1) Influence of air movement.
(2) Influence of age (size) of larvae.
(3) Influence of food previously eaten by larvae, or strain of larvae.

In addition, by virtue of the laboratory set-up used in these experiments, some further conclusions may be drawn which are not completely in accord with some previous conclusions reached by others. One of these further conclusions is with regard to the ability of lepidopterous larvae to "recognize" food plants from a distance. In the present experiments, the larvae were attracted to the plants from a distance of over 120 mm without any difficulty and responded in most cases immediately.

Chin (1950) reports that the larvae of the Colorado potato beetle *(Leptinotarsa)* could not perceive its food plant from a distance farther than 2mm. Gupta and Thorsteinson (1960) tested the olfactory response of the larvae of the diamond backed moth [*Plutella maculipennis* (Curt.)] toward mustard oil odors and found that 5 mm was the maximum distance of response. Dethier (1959) in a study on food plant distribution and density and larval dispersal as factors affecting insect populations, states in relation to field observations that "Larvae find new food-plants by chance alone." It is presumed that he had reference to the gross search for new food plants, not the closer relationship. In the case of the experiments of Chin, and Gupta and Thorsteinson, the screen test was used. In this, the larvae were placed on a screen *above* the food plant or other attractive source. In the present experiments, the larvae were *below* the food plants. This difference could account for a fundamental difference when testing for an odiferous substance heavier than air. Under natural conditions, our observations have always indicated that larvae tend to climb upwards rather than down so that tests involving simulated natural conditions should be best performed with the attraction above rather than below the larvae (Fig. 3). This point will be covered more fully in tests to be described in later papers.

In a paper available to us after the tests here indicated were performed, (Takata, 1959), there has been indicated a change in food plant preference of larva when reared successively on different food

3. Larva of *Pieris rapae* on edge of pot during one of the selection experiments reaching out to black mustard plant three to four inches away. The larva had already traveled several inches under directed impulse to get this far.

plants for several generations. These results complement ours, even though the technique of testing used by Takata was different. He used cabbage and radish with *Pieris rapae crucivora* larvae. In testing, he used actual feeding experiments of the weight of leaves of the plants eaten by the larvae.

SUMMARY

The evidence in this paper indicates (1) that larvae of *Pieris rapae* are capable of detecting their preferred food plant from a distance of at least 120 mm under the conditions of these experiments, (2) that the direction of wind movement is very important to detection of food plant, presumably through odor, (3) that the older and larger larvae are much better at selecting the preferred food plant at least at a distance and (4) that the influence of food previously eaten by the larvae, or the strain of the larvae, directly influences their choice of food in the direction of the previously eaten food.

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